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Macrophage Function in Infectious Disease with Inbred Rabbits

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INTRODUCTION

There is considerable evidence that the inception and progress of tuberculosis have genetic components (27), and that under certain conditions rabbits develop a form of this disease closely resembling what occurs in man. For these reasons, an inbred rabbit colony was begun 33 years ago by the senior author at the Henry Phipps Institute (23).

The most important factor influencing native and acquired resistance to tuberculosis is the ability of macrophages to inhibit the growth of the bacilli within their cytoplasm. This factor determines how many primary tubercles develop after the quantitative inhalation of virulent human-type tubercle bacilli (H37Rv), and the number of such primary tubercles has been used as a criterion for inbreeding for the past 15 years (29).

The history of the rabbits in this colony is summarized in Table 1. They were inbred by brother-sister matings with occasional backcrossing to a parent. Line breeding, i.e., indiscriminate breeding within a given rabbit family, was performed to save families that became infertile by inbreeding. For this reason, the susceptible C family, now in its 21st generation, was line-bred since its 9th generation. The resistant T family in its 11th generation and the rather susceptible AD family in its 11th generation are now also being line-bred. The rather susceptible FCCa family in its 7th generation is still being inbred. These four families represent the major families in our colony today.

The purpose of this report is to list in one place characteristics of past and present members of this rabbit colony and the scientific contributions derived therefrom.

STUDIES CONCERNING TUBERCULOSIS

The use of this inbred rabbit colony has contributed more to the field of tuberculosis than to any other field (Tables 1 and 2). These contributions are summarized in this section.

Resistance to Attack by Tuberculosis

Resistance to attack by an infectious disease is to be contrasted with resistance to its progress (17, 24). By attack we refer to the initial multiplication of an infectious agent in the host; by progress we refer to its continued multiplication in the host. Inapparent infections represent host resistance to progress rather than attack, because in this situation the infectious agent con-

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TABLE 1. *Relative resistance of the inbred rabbit families determined by their survival after a standard intracutaneous inoculation of virulent bovine-type tubercle bacilli (Ravenel) or by their response to the quantitative inhalation of human-type tubercle bacilli (H37Rv), or by both*

Family	Present status[a]	Derivation	Avg survival after infection with bovine bacilli		No. of inhaled human bacilli required to form one tubercle (the "ratio")[b]		Literature references for	
			Days	No. of rabbits	Ratio	No. of rabbits	Bovine bacilli	Human bacilli
Ca	E	Carworth farms (29)	—	—	49 ± 19	9	—	29
C	A	Swift stock (23)	121	5	70 ± 14	30	23	38
CaC	E	Ca × C (3, 38)	—	—	97 ± 12	7	—	27
F	E	Swift stock (23)	141	4	—		23	—
FC	E	F × C (29)	132	8	79 ± 9	45	29	38
FCCa	A	FC × CaC (17)	—	—	30 ± 3	9	—	—[c]
A[d]	E	Stubbs (23)	539	6	—		23	—
AD[d]	A	A × D[e] (30)	197	10	362 ± 103	12	38	38
T(III)	A	Sawin (29)	422	8	1,065 ± 138	71	29	38
TA	E	T × A[d] (37)	—	—	154 ± 27	9	—	37
TTC	A	TC × T (36)	—	—	315 ± 67	12	—	37

[a] A = alive today; E = extinct.

[b] In 1964, six rabbits from each of four rabbit families were exposed to a strain of H37Rv of rather high virulence (13). The "ratios" were as follows: T = 182 ± 36; AD = 56 ± 4; FCCa = 44 ± 3; C = 48 ± 8. The mean ratio and its standard deviation are listed.

[c] Unpublished data.

[d] The resistance of families A and AD has declined since the ninth and seventh generation, respectively (27).

[e] The D family of Swift stock was of intermediate resistance (23).

tinues multiplication long enough to stimulate an immunological response.

Two factors influence resistance to attack by inhaled tubercle bacilli: the trapping of bacilli in the lung, and the initial inactivation of these bacilli.

The trapping of bacilli in the lung seemed to be, in part, dependent on the ability of the alveolar macrophages (AM) to ingest them: AM from certain resistant rabbits were able to ingest twice as many bacilli in vitro in a given period of time as AM from certain susceptible rabbits (17). These findings could explain why resistant rabbits developed tuberculosis sooner than susceptible rabbits when both groups breathed infected air in a closed room over a period of months (24). Highly virulent bovine-type bacilli were used in this experiment. Every one of these is capable of producing a tuberculous lesion in resistant, as well as in susceptible, rabbits when it is trapped in the pulmonary alveoli (30).

In contrast, for every inhaled human-type tubercle bacillus which produced a lesion, many were inactivated (29, 38). Resistant rabbits inactivated more than susceptible rabbits. Thus, in spite of trapping more bacilli, the former resisted attack better than the latter (29). In man, both the human and bovine types seem to be of intermediate virulence. One would therefore expect both the trapping of bacilli and their initial inactivation to influence man's resistance to attack by this disease.

Resistance to the Progress of Tuberculosis

Resistant rabbits lived about twice as long as susceptible rabbits after infection with virulent bovine-type tubercle bacilli (23, 24, 29). In primary pulmonary tubercles, resistant rabbits inhibited more effectively the accumulation or multiplication of bacilli (6, 23, 38), which is influenced by both the rate of bacillary division and the rate at which the bacilli are inactivated. The greater restriction of bacillary accumulation was associated with a more rapid maturation of epithelioid cells in resistant rabbits (21, 38). The spread of bacilli from the pulmonary lesions to the hilar lymph nodes was, however, greater in these animals (6, 38), but the growth of the bacilli once they had reached these nodes was markedly inhibited (6, 23, 38). Conversely, the spread of bacilli to the hilar nodes was less in susceptible animals (6, 38), but, once there, the bacilli multiplied sufficiently to produce caseous lesions, typical of the primary childhood type of tuberculosis (23).

Bacilli invaded the blood stream of both resistant and susceptible rabbits. In early lesions of resistant rabbits, interstitial inflammation

TABLE 2. *Characteristics of resistance and susceptibility to tuberculosis in Phipps rabbits*

Characteristic	Resistance		Susceptibility	
	Degree*	Family and reference	Degree*	Family and reference
Bacilli				
Trapping of tubercle bacilli in lung	++++	A (24); T (6, 17, 38)	++	F (24); C (6, 17, 24, 38); FCCa (17)
Initial inactivation of inhaled bacilli	+++	T (6, 38)	+	C (6, 38)
Subsequent inhibition of bacillary accumulation	++++	T (6, 38)	++	C (6, 38)
Drainage of bacilli to tracheobronchial lymph nodes	++++	T (6, 38)	+	C (6, 38)
Histopathology				
Rate of mobilization of mononuclear cells (in early pulmonary lesions)	+++	T (38)	+	C (38)
Rate of epithelioid cell maturation	++++	A (23); T (29, 32, 38)	+	C (23, 29, 32, 38); F (23)
Pneumonic inflammation	+	T (38)	++++	C (38)
Interstitial inflammation	++++	T (38)	+	C (38)
Maturation of caseous process	++++	T (38)	++	C (38)
Gross pathology				
Number of gross tubercles 5 weeks after inhalation of human bacilli	+	T (29, 32, 38)	++++	C (36, 38); Ca (29); FC (29, 32, 38)
Size of tubercles	++	T (38)	++++	C (38); FC (38)
Cavity of formation	+	A (23); T (38)	±	F (23); C (23, 38)
Spread of disease to kidneys and other organs	+	A (23); T (6, 27)	+++	F (23); C (23)
Rate of healing of lesion	++++	A (23); T (29)	+	F (23); C (23); FC (29)
Other factors				
Amount of acquired immunity	++++	A (23); T (32)	+	F (23); C (23); FC (32)
Longevity after infection with virulent bovine bacilli	++++	A (23, 24); T (29)	+	F (23, 24); C (23, 24); FC (6, 27)

* Symbols: + signifies a low degree; ++++, a high degree; ++ and +++, intermediate degrees.

was more pronounced (38), and more invasion of the blood and lymph occurred. In older lesions of susceptible rabbits, more blood vessels and lymphatics were injured by the caseous process (23), and more bacilli entered these channels (23, 38). Thus, attributes of both resistance and susceptibility contributed to the dissemination of bacilli from the primary lesion. The decisive factor, however, was not the number of bacilli disseminated, but the fate of these bacilli in their new environment. Resistant rabbits inactivated a much larger percentage of bacilli that reach the lymph nodes, spleen, and kidneys than did susceptible rabbits (6, 23, 38).

Pathogenesis of Tuberculosis Caused by Bovine-Type Bacilli

The severity of tuberculosis was determined by both the susceptibility of the host and the virulence of the infecting bacilli. Fully virulent bovine-type bacilli produced one pulmonary lesion for every bacillus that reached the alveolar spaces (30), regardless of the native resistance of the host. In *susceptible* rabbits these bacilli accumulated to a much greater degree than in resistant animals (6, 23, 27), and this accumulation continued, though at a reduced rate, even after acquired immunity developed (6). Caseation necrosis resulted from sensitivity to the tuberculin-like products of the bacilli. It began at the centers of the tubercles and spread centrifugally, destroying the host defenses which attempted to localize the disease (23). Blood vessels and lymphatics became necrotic, and bacilli entered their lumens and spread throughout the body. In the lungs many secondary tubercles developed, which became confluent and killed the host by caseous pneumonia. Cavity formation was rare.

At autopsy, progressive caseous tuberculosis was also present in the tracheobronchial lymph nodes, kidneys, spleen, and bone marrow (23).

In *resistant* rabbits the accumulation of bovine bacilli was more effectively restricted after acquired immunity developed (6, 23, 27). Many of the lesions were epithelioid-cell granulomata, and caseation was less extensive. Secondary hematogenous and lymphogenous lesions in the lungs and other organs were infrequent. The caseous lesions were surrounded and localized by tuberculous granulation tissue, consisting of macrophages, lymphocytes, fibroblasts, capillaries, and lymphatics. In time, fibrous capsules developed around them, and the caseous centers sometimes calcified. More often, however, they underwent liquefaction, a process in which fluid was absorbed and the caseous material softened. In this new medium the bacilli multiplied in tremendous numbers, causing further destruction of tissue. They eroded the peripheral branches of the bronchial tree which they entered, and spread to other parts of the lung, where they produced areas of caseous pneumonia and new lesions which in turn liquefied. The large numbers of bacilli overwhelmed the existing immunity, high as it was, and the ulcerative pulmonary phthisis progressed until death. Without the process of liquefaction, genetically resistant rabbits would have conquered their disease. At autopsy the other organs of the body, including the tracheobronchial lymph nodes, showed few, if any, progressive lesions (23).

Pathogenesis of Tuberculosis Caused by Human-Type Bacilli

Human strains of tubercle bacilli are less virulent for rabbits than the bovine strains. Recovery from infection with them was the rule even in genetically susceptible rabbits. The use of these strains of bacilli has made possible the development of one of the most precise indices of resistance to infection. Briefly, it consisted of quantitatively exposing resistant and susceptible rabbits to an aerosol of bacilli and counting the number of primary pulmonary tubercles which developed 5 weeks later. Since the human strains of bacilli are of reduced virulence for the rabbit, many microorganisms reaching the alveolar spaces were inactivated for every one that multiplied. In other words, because of phenotypic variations among the bacilli aerosolized and phenotypic variations among the alveolar phagocytes which ingested them, only the most virulent bacilli in the aerosol were able to grow in the most susceptible alveolar phagocytes of the host. Resistant rabbits apparently had more resistant phagocytes and fewer susceptible phagocytes than did susceptible rabbits (29). The resistant phagocytes were of two types: those that initially inactivated the bacilli, and those that accomplished this only after acquired immunity developed. Both types of phagocytes would prevent the development of the primary tubercles observed in the lung 5 weeks after infection. Thus, this index of resistance measured a combination of resistance to attack and resistance to progress.

After the inhalation of human-type bacilli, the most resistant rabbits developed about 5% the number of primary tubercles found in the most susceptible rabbits (Table 1). These tubercles were usually smaller and more interstitial in character, and usually contained fewer viable bacilli (38). Their caseous centers were more mature, in that they contained more completely disintegrated nuclear debris (38). In time these caseous centers liquefied (38), but healing was rapid (29) and secondary lesions were rare (29).

In the susceptible rabbits the primary lesions were more intra-alveolar in location and contained far more living bacilli. Their caseous centers showed incomplete digestion of nuclear debris (38). Liquefaction did not occur (38), healing was delayed (29), and secondary lesions were common (27, 29).

BCG Infection

BCG is even less virulent for rabbits than human bacilli. The inhalation of many thousands of bacilli was required to produce a single primary pulmonary tubercle, which was quite small and healed rapidly (S. Abramson, *unpublished data*). Because of the difficulty in finding such pulmonary tubercles, the response of resistant and susceptible rabbits to BCG was best studied after intradermal inoculation. In the skin of resistant rabbits, the BCG nodules grew more rapidly, reached a peak more quickly, ulcerated more frequently, and healed sooner (32). The bacilli multiplied for a shorter time and were subsequently more rapidly inactivated (32). The associated histological responses were also accelerated: epithelioid cells matured faster, and plasma cells and fibroblasts appeared sooner (32). The BCG nodules of susceptible rabbits showed the opposite characteristics. The more rapid growth of the BCG nodule in the resistant animal was associated with a more rapid development of tuberculin sensitivity (32).

The acquired immunity from BCG vaccination was higher in resistant than in susceptible animals (32). At 5 weeks after the inhalation of human-type tubercle bacilli, the number of primary tubercles in resistant rabbits was reduced 80% by BCG vaccination; the number in

susceptible rabbits was reduced only 15%. In other words, BCG vaccination increased the resistance of resistant animals fivefold, and increased the resistance of susceptible animals only 1.2-fold. Thus, vaccination helped the most the rabbits that needed it the least and, conversely, helped the least the rabbits that needed it the most.

These results were not surprising. The ability of the host to inhibit the growth of tubercle bacilli in its tissues is largely due to the immunity acquired by macrophages as a result of the infection. Natively resistant animals acquire more immunity than susceptible animals from either a virulent infection or an attenuated one like BCG. Acquired resistance is therefore superimposed and determined by native resistance.

IMMUNOLOGICAL STUDIES

The immunological attributes studied in this colony of rabbits fell into five categories: (i) macrophage function, (ii) antibody production, (iii) delayed hypersensitivity, (iv) localization of the infection, and (v) white blood cell counts (Table 3).

Macrophage Function

The macrophages of the more resistant rabbits were better able to inactivate the tubercle bacillus in vivo (6, 23, 27, 29, 38), and were more resistant to the cytotoxic effect of tubercle bacilli in vitro (18, 19). Intact oil-induced peritoneal macrophages of certain natively resistant families showed greater dehydrogenase and acid phosphatase activities than did intact macrophages of certain susceptible families (3). When a temporary decrease in acquired resistance was produced in susceptible rabbits by revaccination with BCG (5), certain macrophage dehydrogenase activities were also decreased (4). Frozen and thawed macrophages of various inbred families showed differences in their content of two esterases and an enzyme hydrolyzing *N*-benzoyl-D,L-phenylalanine-β-naphthol ester (12), but no differences were found in their proteinase, lipase, and lysozyme contents (7, 12). Many more macrophage enzymes will have to be studied before an understanding of their contribution to native resistance is achieved.

Antibody Production

Some of the rabbit families, e.g., T and AD, produced high levels of antibodies; others produced only low levels (23, 27, 29, 32). A general correlation between resistance to tuberculosis and antibody production existed, with several exceptions (23). The susceptible C family seemed to produce low antibody titers to bovine γ-globulin and yet produced normal titers to the Lac-hapten of Karush and Saha (*unpublished data*), which is of polysaccharide configuration (20). The titers of bactericidins against *Salmonella typhosa* strain O901 were similar in normal sera from both resistant and susceptible rabbits (L. H. Muschel, *unpublished data*).

Delayed Hypersensitivity

In general, resistant families developed higher degrees of delayed hypersensitivity than did susceptible families after (i) the injection of dead tubercle bacilli (23), (ii) infection with human tubercle bacilli (29), (iii) the intradermal inoculation of BCG (32), or (iv) vaccinia infection (40). The growth of vaccinia virus in the skin was apparently equal in all the families tested, but susceptible strains showed more systemic reaction to intradermal infection, as evidenced by weight loss, debilitation, and occasional death (40).

Localization of the Infection

Localization of infectious agents in the skin has been linked with its protective function. The resistant rabbit families localized intradermally injected tubercle bacilli better than did some susceptible families (23). Unrelated substances such as carbon particles and hemoglobin were also localized better by resistant families (13, 22, 23, 28). The localizing response of the skin was not, however, always correlated with resistance to tuberculosis (13, 22, 23, 28). The dermal response to the lipopolysaccharide PmKo was another variable which was sometimes, but not always, correlated with native resistance to tuberculosis (13). The response to PmKo seemed independent of the skin's localizing response (13).

White Blood Cell Counts

When placed in the cold (5 C), the resistant T rabbits responded with higher polymorphonuclear cell counts in the blood than did susceptible FC rabbits (A. M. Dannenberg, Jr., and M. B. Lurie, *unpublished data*). Under normal conditions, however, no differences among the families were found in total and differential cell counts (Dannenberg and Lurie, *unpublished data*), or in absolute basophil counts (W. B. Shelley, *unpublished data*; 41).

GENETIC STUDIES

Resistance

Genetic studies have been carried out on this colony and are summarized in Table 4. A hybrid rabbit strain (F_1) was obtained by crossing a highly resistant and highly susceptible strain (36). Its resistance to tuberculosis was intermediate

TABLE 3. *Immunological studies with Phipps rabbits*

Subject	Family characteristics and literature reference
Macrophage function	
Ability of macrophages to inactivate tubercle bacilli in vivo	Efficient: T (6, 29, 38), A (23); intermediate: AD (37); inefficient: C (6, 29, 38); F (23); FC (29)
Ability of macrophages to resist injury by tubercle bacilli in vitro	High: T (18); low: FCCa (18, 19)
Rate of macrophage mobilization by mineral oil intraperitoneally	T, AD, and FC: no apparent difference (12) lower for T than for TTC and CaC (3)
Macrophage enzymes	
Dehydrogenases for glycerophosphate and β-hydroxybutyrate	High: T (3); intermediate: TTC (3); low: CaC (3)
Dehydrogenases for glucose-6-phosphate and glyceraldehyde-3-phosphate	High: T (3); low: CaC (3)
Acid phosphatase	High: T (3); low: CaC (3)
BPNase (substrate: *N*-benzoyl-D,L-phenylalanine-β-naphthol ester)	Normal: T (12), AD (12); low: FC (12)
Esterases hydrolyzing methyl butyrate	High: AD (12); intermediate: T (12); low: FC (12)
β-Naphthyl acetate	High: FC (12); intermediate: T and AD (12)
Proteinase, lipase, and lysozyme	T, AD, and FC: no apparent difference (7, 12)
Antibody production	
In tuberculosis	High: T (29, 32) A (23); intermediate: D (23); low: C (29), FC (32), F (23)
To bovine serum albumin	High: AD (31); intermediate: T (31); low to intermediate: FC (31)
To bovine γ-globulin	High: AD (Saha, and Karush, *unpublished data*); intermediate: T and FCCa (Saha and Karush, *unpublished data*); low: C (Saha and Karush, *unpublished data*)
To haptens: *Lac* of Karush (polysaccharide in configuration) (20)	High: AD (Saha and Karush, *unpublished data*); intermediate: T, FCCa, and C (Saha and Karush, *unpublished data*)
Bactericidins against *Salmonella typhosa* O901 in normal serum	No differences between T, AD, FCCa and C (Muschel, *unpublished data*)
Delayed sensitivity	
To heat-killed tubercle bacilli	High: A (23); intermediate: B (23); low: F (23)
To live tubercle bacilli	Rapid: T (29, 32); slow: C (29, 32); FC and Ca (29)
To vaccinia virus	Rapid: T and AD (40); slow: C, CaC, and FCCa (40)
Localization in skin	
Tuberculosis	Excellent: A (23); poor: F and C (23)
Carbon particles	Excellent: A (23) and T (13); poor: F (23) and C (22, 23)
Hemoglobin	Excellent: T and AD (13); poor: FCCa and C (13)
PmKo	T: large reaction (13); C and FCCa: intermediate reaction (13) AD: small reaction (13)
Blood cell counts	
Total white cell counts	T and C: no differences (Dannenberg and Lurie, *unpublished data*)
Differential cell counts	T and C: no differences (Dannenberg and Lurie, *unpublished data*)
Absolute basophil counts	T, AD, FCCa, and C: no differences (Shelley, *unpublished data*)

TABLE 4. *Genetic studies with Phipps rabbits*

Subject	Family characteristics and literature reference
Resistance to tuberculosis	T × C = TC (intermediate) (36); TC × T = TTC (as resistant as T) (36); TC × C = TCC (intermediate between TC and C) (36)
Transplantation antigens	C skin grafted on C rabbits survived an average of 30 days; controls averaged 12 days (11)
Lymph node cell transplantation	Lymphoid cells from C rabbits vaccinated with *Shigella* antigens survived longer in recipient C rabbits than in random-bred rabbits (maximal agglutinin titers were seven times controls) (Harris and Ogburn, *unpublished data*)
Color	T, C—Albino (homozygous); AD, FC, CaC, FCCa—Dutch (heterozygous); A—English (heterozygous)
Blood types*	See nested table below
γ-Globulin allotypes†	See nested table below

Rabbit family	Blood types	Segregating blood type
T	A, D, E, L, M	A–D; E
C	C, F, L, M	C–L
FCCa	A, B, C, F	A–F
CaC	B, C, F, M	B–M
AD	A, C, M	None

T—A_a^3/A_a^3, A_b^4/A_b^4	Homozygous
TA—A_a^3/A_a^3, A_b^4/A_b^4	Homozygous
T^3F—A_a^3/A_a^3, A_b^4/A_b^4	Homozygous
C—A_a^2/A_a^2, A_b^4/A_b^4	Homozygous
A—A_a^2/A_a^2, A_b^4/A_b^4	Homozygous
FCCa—A_a^2/A_a^2, A_b^4/A_b^4	Heterozygous in *a* locus; homozygous in *b* locus
A_a^2/A_a^3, A_b^4/A_b^4	
A_a^3/A_a^3, A_b^4/A_b^4	
CaC—A_a^2/A_a^3, A_b^4/A_b^4	Heterozygous in *a* locus; homozygous in *b* locus
A_a^3/A_a^3, A_b^4/A_b^4	
AD—A_a^2/A_a^2, A_b^4/A_b^4	Heterozygous in *a* locus; homozygous in *b* locus
A_a^1/A_a^2, A_b^4/A_b^4	

* Cohen, *unpublished data.*
† Dray, *unpublished data.*

between that of each parent strain. The backcross of the F_1 hybrid generation to the *resistant* ancestors produced F_2 strains of the same high resistance as the original resistant strain. The backcross of the F_1 hybrid to susceptible ancestors produced F_2 strains that were more resistant than the original *susceptible* strain. Factors determining resistance were therefore either more dominant in the phenotype than determinants for susceptibility, or susceptible individuals lacked certain qualities which resistant individuals possessed. Determinants for resistance to tuberculosis were multiple, complex, and additive (36). Similar studies in mice have been recently published (39).

Transplantation

Skin transplantation between members of the C family showed a two- to threefold prolongation of skin rejection time (11), indicating partial homozygosity to transplantation antigens. The C family was in its 19th generation. Certain families at the Jackson Laboratory in Maine (the Y-line of C. K. Chai) were only in their 12th generation, and yet exchanged skin grafts survived an average of 56 days (8). Two grafts survived over 200 days (8). Our C family was inbred for susceptibility to tuberculosis; Chai's Y-line was apparently inbred for skin transplantation. Our C family was homozygous at both the *a* and *b* loci for γ-globulin (S. Dray, *unpublished data*). Chai's Y-line was homozygous at the *a* locus, but not at the *b* (Dray, *unpublished data*). The C family, which is only a fair producer of antibodies (29; Saha and Karush, *unpublished data*) and delayed hypersensitivity (29, 32), seemed to reject heterologous skin grafts at approximately the normal rate (11).

Popliteal lymph node cells from C rabbits immunized with *Shigella* antigens were transplanted into (i) C rabbits and random-bred rabbits, and (ii) irradiated C rabbits and irradiated random-bred rabbits (T. N. Harris and C. A. Ogburn, *unpublished data*; 16). In each case, the agglutinin titers of the C recipients were higher than those of random-bred controls. In other words, the transplanted "C" lymphoid cells lived longer and produced more antibody in the inbred C recipients.

Genetic Markers

Additional criteria for inbreeding are such genetic markers as color, blood types, and γ-globulin allotypes. The T and C families are true albinos. CaC, FC, FCCa, and AD manifest Dutch characteristics, and the A family had English markings (23). All the families tested except the AD were heterozygous in their blood types (C. Cohen, *unpublished data*; 9, 10; Table 4). The T and C families were homozygous in their γ-globulin allotypes, whereas CaC, AD, and FCCa families were heterozygous (Dray, *unpublished data*; 14, 15; Table 4).

Endocrinological Studies Involving Thyroid and Adrenal Function

These studies were of two types: (i) the effect of an altered endocrine balance on host response to the tubercle bacillus, and (ii) the identification of differences in endocrine balance existing among these inbred strains (Table 5).

In the first study, rabbits under the influence of cortisone (25, 26, 33, 35) or antithyroid drugs (26, 31), and rabbits that had been thyroidectomized (26, 31), responded to the inhalation of human-type tubercle bacilli as genetically susceptible rabbits. They had a greater number of primary pulmonary tubercles than did controls, and these tubercles resided more intra-alveolarly and had less interstitial inflammation surrounding them. More bacilli proliferated in their lesions and fewer drained to the hilar lymph nodes. The maturation of epithelioid cells was delayed in these rabbits, and the disintegration of the nuclear debris in the caseous centers of their tubercles was incomplete. Conversely, rabbits under the influence of triiodothyronine or thyroxine responded to the inhalation of tubercle bacilli as genetically resistant rabbits (37). They had fewer primary tubercles with more interstitial inflammation, fewer bacilli in their lesions with more bacilli reaching the hilar lymph nodes, and faster maturation of both epithelioid cells and the caseous process.

The spread of tuberculosis from an intracutaneous site was markedly restricted in rabbits of the C family by the administration of estrogen (28), and enhanced in the A family by chorionic gonadotropin (28). Estrogen significantly suppressed the development of amyloid degeneration

TABLE 5. *Endocrinological studies with Phipps rabbits*

Subject	Family characteristics and literature reference
Adrenals	Small in FC (34)
Cortisone reduced resistance to tuberculosis	In FC, Ca, and TTC (34)
Thyroid hormones increased resistance to tuberculosis	In AD, TTC, TA, and CaC, but not in T and C (37)
Thyroidectomy and goitrogens reduced resistance to tuberculosis	In T, TA, and CaC (26, 31)
Estrogen restricted the spread of cutaneous tuberculosis	In C (28)
Estrogen suppressed the development of amyloid degeneration in tuberculous rabbits	In A and C (28)
Chorionic gonadotropin increased the spread of cutaneous tuberculosis	In A (28)
Hyperthyroidism increased the number of oil-induced peritoneal exudate cells, and increased the activities of several of their dehydrogenases	In TTC (1) and AD (1)
Hyperthyroidism increased phagocytosis of carbon particles by the reticuloendothelial system and hypothyroidism decreased it	In AD (1)
Cortisone decreased the number of oil-induced peritoneal exudate cells, increased their protein content, and tended to decrease their acid phosphatase activity and the activities of several of their dehydrogenases	In TA (2) and CaC (2)

in tuberculous rabbits of both of these strains (28).

Altered adrenal or thyroid function produced changes in the number of macrophages, induced by the intraperitoneal injection of mineral oil, and changes in the activities of certain of their enzymes when intact cells were tested (1, 2).

In the second study, the FC family was characterized by small adrenals (34), and in one experiment the resistance of these rabbits to tuberculosis seemed to be improved by a small dose of adrenocorticotrophic hormone (33). Under the influence of thyroid hormone, the T and C families did not become more resistant to tuberculosis, whereas the AD, TTC, TA, and CaC families did (37).

CONCLUSIONS AND OUTLOOK

Members of the Phipps colony of rabbits have been inbred for resistance or susceptibility to tuberculosis for a third of a century. The use of these rabbits has contributed considerably to the experimental pathology of allergic and infectious diseases. In the last analysis this colony has been inbred for macrophage function, because these cells are mainly responsible for the inactivation of tubercle bacilli. Macrophages, however, like every other cell of the host, are controlled by numerous interacting and integrative variables, which in this case are of a genetic, immunological, and endocrinological nature. The data emphasize the interdependence and organization of the innumerable phenomena which constitute life.

As for the future, many problems remain to be solved. What is the exact nature of cellular immunity? How much is specific and how much nonspecific? The roles of cell proliferation, cytophilic antibodies, and the production, stability, and enzyme and bactericidin content of lysosomes need further investigation. What is the cause of caseation and liquefaction? Is it hypersensitivity, autolytic enzymes, bacterial products, or combinations of these? Can pharmacological products be developed to enhance native and acquired resistance and eliminate tissue destruction in tuberculosis and other allergic and infectious diseases? Since man has a long way to go in the solution of these basic problems, this inbred rabbit colony should continue to be of use in many experiments yet to be performed.

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LITERATURE CITED

1. ALLISON, M. J., AND E. GERSZTEN. 1962. Metabolic studies on mononuclear cells from rabbits of varying genetic resistance to tuberculosis. III. Studies on variation of thyroid function. Am. Rev. Respirat. Diseases **86**:513–517.
2. ALLISON, M. J., AND E. GERSZTEN. 1963. Metabolic studies on mononuclear cells from rabbits of varying resistance to tuberculosis. IV. Studies on cortisone-treated animals. Am. Rev. Respirat. Diseases **87**:384–388.
3. ALLISON, M. J., P. ZAPPASODI, AND M. B. LURIE. 1961. Metabolic studies on mononuclear cells from rabbits of varying genetic resistance to tuberculosis. I. Studies on cells of normal noninfected animals. Am. Rev. Respirat. Diseases **84**:364–370.
4. ALLISON, M. J., P. ZAPPASODI, AND M. B. LURIE. 1962. Metabolic studies on mononuclear cells from rabbits of varying genetic resistance to tuberculosis. II. Studies on cells from BCG-vaccinated animals. Am. Rev. Respirat. Diseases **85**:364–372.
5. ALLISON, M. J., P. ZAPPASODI, AND M. B. LURIE. 1962. The correlation of a biphasic metabolic response with a biphasic response in resistance to tuberculosis in rabbits. J. Exptl. Med. **115**:881–890.
6. ALLISON, M. J., P. ZAPPASODI, AND M. B. LURIE. 1962. Host-parasite relationships in natively resistant and susceptible rabbits on quantitative inhalation of tubercle bacilli. Am. Rev. Respirat. Diseases **85**:553–569.
7. CARSON, M. E., AND A. M. DANNENBERG, JR. 1965. Hydrolytic enzymes of rabbit mononuclear exudate cells. II. Lysozyme: properties and quantitative assay in tuberculous and control inbred rabbits. J. Immunol. **94**:99–104.
8. CHAI, C. K. 1964. Skin grafts between inbred rabbits. Transplantation **2**:436–437.
9. COHEN, C. 1962. Blood groups in rabbits. Ann. N.Y. Acad. Sci. **97**:26–36.
10. COHEN, C., AND R. G. TISSOT. 1965. Blood groups in the rabbit. Two additional isoantibodies and the red cell antigens they identify. J. Immunol. **95**:148–155.
11. COLBERG, J. E., AND A. M. DANNENBERG, JR. 1965. An evaluation of inbred rabbit popula-

tions by skin homotransplantation. Nature **207**:777–778.

12. DANNENBERG, A. M., JR., AND W. E. BENNETT. 1963. Hydrolases of mononuclear exudate cells and tuberculosis. Arch. Pathol. **76**:581–591.
13. DANNENBERG, A. M., JR., K. MIZUNOE, M. PEACE, AND P. ZAPPASODI. 1965. Dermal response to the lipopolysaccharide PmKo from tubercle bacilli as an index of resistance to tuberculosis. Bull. Johns Hopkins Hosp. **117**:174–194.
14. DRAY, S., S. DUBISKI, A. KELUS, E. S. LENNOX, AND J. OUDIN. 1962. A notation for allotypy. Nature **195**:785–786.
15. DRAY, S., G. O. YOUNG, AND L. GERALD. 1963. Immunochemical identification and genetics of rabbit γ-globulin allotypes. J. Immunol. **91**:403–415.
16. HARRIS, S., T. N. HARRIS, C. A. OGBURN, AND M. B. FARBER. 1956. Studies on the transfer of lymph node cells. VII. Transfer of cells incubated *in vitro* with filtrates of trypsin-treated suspensions of *Shigella paradysenteriae*. J. Exptl. Med. **104**:645–661.
17. HENDERSON, H. J., A. M. DANNENBERG, JR., AND M. B. LURIE. 1963. Phagocytosis of tubercle bacilli by rabbit pulmonary alveolar macrophages and its relation to native resistance to tuberculosis. J. Immunol. **91**:553–556.
18. HSU, H. S. 1965. *In vitro* studies on the interactions between macrophages of rabbits and tubercle bacilli. I. Cellular basis of native resistance. Am. Rev. Respirat. Diseases **91**:488–498.
19. HSU, H. S. 1965. *In vitro* studies on the interactions between macrophages of rabbits and tubercle bacilli. II. Cellular and humoral aspects of acquired resistance. Am. Rev. Respirat. Diseases **91**:499–509.
20. KARUSH, F. 1957. The interaction of purified anti-β-lactoside antibody with haptens. J. Am. Chem. Soc. **79**:3380–3384.
21. LURIE, M. B. 1932. The correlation between the histological changes and the fate of living tubercle bacilli in the organs of tuberculous rabbits. J. Exptl. Med. **55**:31–54.
22. LURIE, M. B. 1938. Nature of inherited resistance to tuberculosis. Proc. Soc. Exptl. Biol. Med. **39**:181–187.
23. LURIE, M. B. 1941. Heredity, constitution and tuberculosis: an experimental study. Am. Rev. Tuberc. **44** (suppl. to No. 3):1–125.
24. LURIE, M. B. 1944. Experimental epidemiology of tuberculosis. Hereditary resistance to attack by tuberculosis and to the ensuing disease and the effect of the concentration of tubercle bacilli upon these two phases of resistance. J. Exptl. Med. **79**:573–589.
25. LURIE, M. B. 1955. On the role of hormones in experimental tuberculosis, p. 18–48. *In* Advances in tuberculosis research, vol. 6. S. Karger, Basel.
26. LURIE, M. B. 1960. The reticuloendothelial system, cortisone and thyroid function: their relation to native resistance to infection. Ann. N.Y. Acad. Sci. **88**:83–98.
27. LURIE, M. B. 1964. Resistance to tuberculosis: experimental studies in native and acquired defensive mechanisms. Harvard Univ. Press, Cambridge, Mass.
28. LURIE, M. B., S. ABRAMSON, A. G. HEPPLESTON, AND M. J. ALLISON. 1949. Constitutional factors in resistance to infection. III. On the mode of action of estrogen and gonadotropin on the progress of tuberculosis. Am. Rev. Tuberc. **59**:198–218.
29. LURIE, M. B., S. ABRAMSON, AND A. G. HEPPLESTON. 1952. On the response of genetically resistant and susceptible rabbits to the quantitative inhalation of human-type tubercle bacilli and the nature of resistance to tuberculosis. J. Exptl. Med. **95**:119–134.
30. LURIE, M. B., A. G. HEPPLESTON, S. ABRAMSON, AND I. B. SWARTZ. 1950. An evaluation of the method of quantitative airborne infection and its use in the study of the pathogenesis of tuberculosis. Am. Rev. Tuberc. **61**:765–797.
31. LURIE, M. B., P. ZAPPASODI, R. G. BLAKER, AND R. S. LEVY. 1959. On the role of the thyroid in native resistance to tuberculosis. II. The effect of hypothyroidism. The mode of action of thyroid hormones. Am. Rev. Respirat. Diseases **79**:180–198.
32. LURIE, M. B., P. ZAPPASODI, E. CARDONA-LYNCH, AND A. M. DANNENBERG, JR. 1952. The response to the intracutaneous inoculation of BCG as an index of native resistance to tuberculosis. J. Immunol. **68**:369–387.
33. LURIE, M. B., P. ZAPPASODI, A. M. DANNENBERG, JR., AND E. CARDONA-LYNCH. 1953. The effect of cortisone and ACTH on the pathogenesis of tuberculosis. Ann. N.Y. Acad. Sci. **56**:779–792.
34. LURIE, M. B., P. ZAPPASODI, A. M. DANNENBERG, JR., AND E. CARDONA-LYNCH. 1953. Constitutional factors in resistance to infection; the effect of cortisone on the pathogenesis of tuberculosis, p. 84–99. *In* G. Shwartzman [ed.], The effect of ACTH and cortisone upon infection and resistance. Columbia Univ. Press, New York.
35. LURIE, M. B., P. ZAPPASODI, A. M. DANNENBERG, JR., AND I. B. SWARTZ. 1951. Constitutional factors in resistance to infection: The effect of cortisone on the pathogenesis of tuberculosis. Science **113**:234–237.
36. LURIE, M. B., P. ZAPPASODI, A. M. DANNENBERG, JR., AND G. H. WEISS. 1952. On the mechanism of genetic resistance to tuberculosis and its mode of inheritance. Am. J. Human Genetics **4**:302–314.
37. LURIE, M. B., P. ZAPPASODI, R. S. LEVY, AND R. G. BLAKER. 1959. On the role of the thy-

roid in native resistance to tuberculosis. I. The effect of hyperthyroidism. Am. Rev. Respirat. Diseases **79**:152–179.
38. LURIE, M. B., P. ZAPPASODI, AND C. TICKNER. 1955. On the nature of genetic resistance to tuberculosis in the light of the host-parasite relationships in natively resistant and susceptible rabbits. Am. Rev. Tuberc. Pulmonary Diseases **72**:297–323.
39. LYNCH, C. J., C. H. PIERCE-CHASE, AND R. DUBOS. 1965. A genetic study of susceptibility to experimental tuberculosis in mice infected with mammalian tubercle bacilli. J. Exptl. Med. **121**:1051–1070.
40. PINCUS, W. B., J. A. FLICK, AND T. H. INGALLS. 1963. The response of inbred rabbits to vaccinia virus infection. J. Immunol. **91**:58–64.
41. SHELLEY, W. B., AND H. M. PARNES. 1965. The absolute basophil count. J. Am. Med. Assoc. **192**:368–370.